Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

DATA ON A PECULIAR MENDELIAN RATIO IN DROSOPHILA AMPELOPHILA

JOSEPH LIFF [1]

A MUTANT with pink eyes was found by Professor T. H. Morgan in the summer of 1910, in one of his culture bottles which contained wild, red-eyed *Drosophila*. He described it as follows:[2]

"The pink eye is more translucent than the red eye, but of about the same general tone. It lacks the dark fleck seen in the red and vermilion eye when the eye is examined with a lens. This black fleck changes its position as the lens travels over the eye. The pink eye, P, is with a little experience easily distinguished from the other colors, especially in newly hatched flies. When the fly gets old the eye turns to a brown color very characteristic of this type of eye."

Pink was found to be recessive to red. The Mendelian expectation in the F_2, viz., three red to one pink, gave the following (Morgan, 1911):

TABLE I

	F_1	F_2		Proportion of Red : Pink
		Red	Pink	
Red ♀ × Pink ♂.........	All red	3,063	169	18 : 1
Pink ♀ × Red ♂.........	All red	1,133	237	5 : 1

The expectation in either case was 3:1, but the numbers realized were 18:1 and 5:1.

In the spring of 1912 I repeated this experiment under the direction of Professor Morgan in order to find whether or not the above ratio would persist. The results have already been published (Morgan, 1912), but a brief summary is here reproduced for reference:

---

[1] From the Zoological Laboratory of Columbia University.

[2] *Jour. Exp. Zoology,* Vol. 11, No. 4, November, 1911.

TABLE II

Red ♀ × Pink ♂ → in F_2				Pink ♀ × Red ♂ → in F_2			
Bottle	Red	Pink	Proportion of Red: Pink	Bottle	Red	Pink	Proportion of Red : Pink
A....	318	94	3.3 : 1	*a*....	541	124	4.3 : 1
B....	140	34	4.1 : 1	*b*....	199	63	3.2 : 1
C....	375	70	5.3 : 1	*c*....	582	136	4.3 : 1

The above are records of mass cultures. When pairs were used, the fluctuations in ratio were much more marked. The records of 40 pairs gave an almost unbroken series running from 1.8:1 up to 6:1. In seven cases out of the 40 (18 per cent.) the pink flies exceeded the expectation; 3 pairs (7.5 per cent.) gave a 3:1 ratio, while in the remaining 30 pairs (75 per cent.) the pink fell behind. The total number produced by these 40 pairs was 4,056, of which 891 were pink—an average ratio of 3.58:1, about the same as that shown in Table II.

In a second experiment the F_1 hybrids were backcrossed to the pink. The expectation was 1:1. But the records of 15 bottles of mass culture showed fluctuations running from 1:1 up to 2.3:1. The total number counted in these back crosses was 5,527, of which 2,391 were pink, giving an average ratio of 2.31:1. The pink flies fell behind again, and in about the same proportion as in the normal cross.[3]

These remarkable fluctuations were observed at the time the experiments were in progress, and it was suggested that some environmental condition was responsible for the results by either accelerating or retarding[4] the development of the one or of the other variety. The fact that all these experiments were performed at the same time, and the bottles kept side by side in a room in which a nearly constant temperature was maintained throughout the winter, precludes the chance of a factor outside the culture bottles operating here. Attention was therefore directed to the condition of the food inside the bottles. An examination seemed to indicate that those in which the food was dry yielded the higher pink ratios. To test this, two of the bottles in which conditions were normal, and in which the F_2 had just begun to emerge, were made "wet" by the addition of a considerable amount of banana juice. But they still showed a similar tendency to yield a relatively higher proportion of pink.

---

[3] For a detailed account of these experiments see Morgan, 1912.

[4] It should be noted here that owing to the danger of overlapping of generations, the bottles were discarded on the tenth day (counting from the day the first F_2 emerged) regardless of the number of unhatched pupæ

To ascertain more definitely whether or not moisture or dryness affected in any way the development of these flies, a special experiment was arranged in which some flies were bred in "dry" bottles, and some in "wet" bottles. In the first case, the banana was thoroughly dried by means of filter paper which was discarded after it had absorbed all the available moisture, and the banana wrapped in fresh paper; in the second, banana juice was added every second or third day, so that there was throughout the experiment an abundant amount of wet food in the bottles. The effect of this treatment is shown in Tables III and IV:

TABLE III

RECORD OF F_2 OF A CROSS RED BY PINK IN WHICH THE FLIES DEVELOPED IN BOTTLES IN WHICH THE FOOD WAS "DRY"

Pink ♀ × Red ♂						Red ♀ × Pink ♂					
Bottle	Red		Pink		Proportion Red : Pink	Bottle	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	95	86	25	18	4.2 : 1	*a*....	91	109	28	32	3.3 : 1
B.....	73	78	20	21	3.7 : 1	*b*....	76	65	29	25	2.6 : 1
C.....	182	164	39	44	4.3 : 1	*c*.....	43	42	14	15	3.0 : 1
D....	99	89	39	42	2.3 : 1	*d*....	161	117	55	46	2.7 : 1

TABLE IV

RECORD OF F_2 OF A CROSS RED BY PINK IN WHICH THE FLIES DEVELOPED IN BOTTLES IN WHICH THE FOOD WAS "WET" FROM THE BEGINNING

Pink ♀ × Red ♂						Red ♀ × Pink ♂					
Bottle	Red		Pink		Proportion Red : Pink	Bottle	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	91	90	27	38	2.8 : 1	*a*....	82	89	17	17	5.0 : 1
B.....	21	26	12	12	2.0 : 1	*b*....	14	15	8	6	2.0 : 1
C....	84	83	22	23	3.7 : 1	*c*.....	21	29	2	6	6.0 : 1

TABLE V

RECORD OF 14 ♀'S, 7 RED AND 7 PINK, BRED IN 7 BOTTLES EACH OF WHICH CONTAINED ONE RED AND ONE PINK FERTILIZED FEMALE. THE TABLE SHOWS THEIR RELATIVE PRODUCTIVITY

	Counted on	Bottle *a*				Bottle *b*				Bottle *c*				Bottle *d*				Bottle *e*				Bottle *f*				Bottle *g*			
		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Hatched from deposits of April 15–20	Ap. 25	7	4	0	0	3	3	0	0	5	3	0	0	20	12	0	0	15	10	0	0	16	7	3	0	Fertilized by sterile ♂	...	14	0
	26	9	9	0	0	12	8	5	0	7	7	0	0	3	5	0	0	2	6	0	0	18	8	0	6		...	11	9
	27	2	9	8	4	1	8	12	15	7	3	3	1	1	0	1	0	7	12	1	2	4	4	0	0		...	5	8
	29	5	5	9	8	7	5	5	5	20	5	3	2	...	...	...	...	11	15	1	1	7	8	2	5		...	5	6
	30	7	7	7	6	2	5	6	5	12	9	3	2	...	...	...	...	9	10	2	0	14	10	7	6		...	6	3
	May 1	4	4	12	14	3	4	4	8	14	12	3	9	...	...	...	...	9	14	5	0	18	15	8	10		...	5	8
	2	16	15	12	11	13	11	7	12	1	3	1	1	...	...	...	...	11	8	2	1	6	4	6	4		...	14	6
	3	12	27	11	13	20	18	11	7	...	...	...	1	...	...	...	...	20	12	6	6	0	0	1	2		...	4	9
	4	1	2	2	3	5	12	2	4	...	...	...	...	...	...	...	...	31	18	4	1	...	...	...	...		...	17	17
	6	...	...	...	...	2	0	0	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...		...	...	...
Hatched from deposits of April 20–25	Ap. 30	3	3	0	0	15	3	0	0	...	...	...	...	13	1	0	0	4	0	0	0	Killed Ap. 20				0	2	0	0
	May 1	4	2	5	7	15	4	9	3	2	0	0	0	18	7	0	0	6	9	7	12	...	...	...	...	10	9	2	0
	2	0	0	5	5	7	5	2	1	1	4	0	0	8	6	2	0	4	3	6	11	...	...	...	...	4	5	2	4
	3	2	1	4	3	14	11	4	3	2	5	0	0	0	0	0	0	10	8	20	20	...	...	...	...	15	6	9	18
	4	0	2	8	11	8	7	1	2	3	0	1	1	0	0	0	0	1	1	5	7	...	...	...	...	16	13	13	19
	6	died Ap. 23	...	1	6	25	20	2	0	13	6	0	1	0	0	1	0	6	0	8	2	...	...	...	...	20	19	14	20
	8		...	2	0	16	17	4	4	7	5	0	0	0	0	1	0	17	15	15	16	...	...	...	...	4	6	13	7
	9		...	...	...	13	15	6	5	6	7	0	0	...	...	...	...	4	6	13	7	...	...	...	...	5	2	8	4
	10		...	...	...	6	6	4	7	6	2	0	0	...	...	...	...	0	4	0	2	...	...	...	...	2	6	6	4
	11		...	...	...	0	1	2	2	0	1	0	0	...	...	...	...	0	2	0	1	...	...	...	...	...	...	...	...
	Total....	72	90	92	101	187	163	86	85	106	72	14	18	63	31	5	0	167	153	95	89	83	56	27	33	76	68	148	142
																						Deduct bottle 1....				...	...	81	66
																										76	68	67	76

It is evident that neither dryness nor moisture has any effect in rendering more favorable the conditions needed for the emergence of either the red or the pink variety. The results show that under unfavorable conditions, large numbers of larvæ and pupæ fail to develop, since the total yield of each and every bottle is far below the normal output; and those which do emerge are but chance survivals.

It was suggested that the reason the pink flies fell behind the expected ratio, was the fact that the mutant was weaker than the wild stock and therefore less likely to come through the larval and pupal stages. If this were the case, they should always fall behind. In many cases, however, they actually exceeded the expectation. Furthermore, they always seemed to be just as vigorous, and to live as long as the wild fly.

The hypothesis which was formed at this stage, and which determined to a large extent the experiments which followed was, that another factor not related to eye-color was at work. Such a factor, if one is assumed to be present, by its independent action might be responsible for the disturbance in ratio. It might, moreover, be present in the wild stock which originally gave rise to the pink, since the wild fly is similarly, though less frequently, affected. It is with the search for such factors that the subsequent experiments will chiefly deal.

Before presenting the data, it will be well to point out some of the possible sources of error which were to a great extent eliminated.

I. The method usually employed in these experiments is as follows: The flies, which are to be cross-bred, are taken out of the culture bottles as soon as they hatch and before they have time to mate. They are then put into a clean, sterilized bottle in mass cultures of about five or more pairs. There they remain till their offspring (F_1) are ready to emerge: 9–10 days in summer and 11–12 or 13 days in winter when the temperature is low. The F_1 flies are placed in fresh bottles for a similar length of time, and then removed. During the succeeding ten days the F_2 are counted each day as they hatch. The bottle is then discarded for fear of overlapping of generations; for, the F_2 might mate and deposit eggs before removal.

It has, however, been observed that each time a bottle is discarded a considerable number of pupæ and even larvæ remain behind. This being the case, it is possible that the ratio we get does not always represent what actually happens. In order to count the total output, it was decided to transfer the flies to a second bottle on the fifth day. All eggs deposited, during the five days that the parent remained there, would thus have at least fifteen days to develop. It was hoped that, in this way, a more representative ratio would be obtained.

II. It was shown (Tables III and IV) that large numbers of larvæ fail to develop when a bottle becomes too "dry" or too "wet." Considerable care was taken to avoid either of these conditions. If a bottle showed a tendency to dry up, fresh food was immediately added; when it was too wet, the moisture was absorbed by filter paper.

III. The yield of a mass-culture bottle is always relatively small as compared with that of the same number of flies mated in pairs. This would indicate probably greater mortality due to overcrowding. For this reason only pairs were used in the later experiments.

In the first of these experiments pure stocks, both pink and red, were used; for it was believed that if differences existed other than the red-pink distribution, between the two varieties, they would be more emphasized if hybridization had not been effected. The chief purpose, however, was to become familiar with the modes of behavior of the races. The experiment follows:

A number of flies, both pink and red, were isolated within one to six hours after hatching and the sexes kept apart for 3–4 days, after which time they were mated, red to red, pink to pink. Immediately after mating, which took place within five minutes to two hours, the males were removed. One red and one pink of these females were put into each of seven bottles. In this way the same environmental conditions were secured for the eggs of both. Five days later they were allowed to mate again (not by the same males) and placed in a second set of bottles. The same two females that were together in the first set were also together in the second. There they remained five more days. Counts of the flies that hatched were made from day to day, and the bottles were emptied as long as they continued to yield. The results are given in Table V.

The records of these flies show several interesting and suggestive facts. It will be noted, in the first place, that the length of larval life varies through wide limits. Each bottle contained eggs which were deposited during a period of no more than five days. The hatching periods, however, extended through eleven days in the first set of bottles (April 25–May 6) and twelve days in the second set (April 30–May 12). The flies which emerged first consumed but ten days for development; those which emerged last took at least sixteen days. This phenomenon was more marked where the number produced was larger, suggesting that crowding may retard the development of some individuals.

Of equal interest is the fact that the pink flies invariably began to hatch from 24 to 48 hours later than the red. This was true in the second set of bottles as well as in the first, which proves that it was not due to late maturity of the parents, for, at the time of transfer, they were in the midst of their productive period.

Another point of interest is to be found in the fact that the pink stock was, on the whole, less fertile than the red. In the two bottles '*a*' and '*g*' where the productivity of the two was about equal the red, like the pink, were also low-producers. This is significant, and will be referred to later.

In order to test the above-mentioned facts, the following experiment was performed. F_1 hybrids were mated in pairs, and transferred during a period of twenty days (May 16–June 4) 13 times, remaining in each bottle from one to two days. Care was taken to count every fly of the F_2 that hatched. The result follows:

TABLE VI

RECORD OF F_2 FLIES WHICH HATCHED FROM EGGS DEPOSITED DURING A PERIOD OF 20 DAYS; DURING WHICH TIME THE PARENTS WERE TRANSFERRED THIRTEEN TIMES

Pink ♀ × Red ♂ → in F_2						Red ♀ × Pink ♂ → in F_2					
Pair	Red		Pink		Proportion Red : Pink	Pair	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	235	209	90	95	2.3 : 1	*a*....	134	118	42	37	3.2 : 1
B.....	220	213	84	75	2.7 : 1	*b*....	105	108	67	64	1.6 : 1
C.....	162	168	65	58	2.7 : 1	*c*.....	189	153	52	53	3.2 : 1
D....	202	119	14	19	9.7 : 1	*d*[5]....	70	58	11	8	6.5 : 1

Here we have a group in which the pink ran sometimes relatively ahead of the red. But the other extreme is also represented in pairs *D* and *d*. The numbers obtained are in each case large enough to be significant.

This experiment was repeated on a larger scale in the fall of 1912. Ten pairs were used for each of these crosses and they were continually transferred as long as they lived. The records follow:

TABLE VII

F_2 RECORD FROM RED-EYED FLIES CROSSED TO PINK-EYED FLIES MATED IN PAIRS. SHOWING THE TOTAL OUTPUT OF EACH F_1 PAIR DURING ITS LIFETIME

PINK ♀ × RED ♂ → IN F_2

Pair	No. of Days Each Pair Lived[6]	No. of Times Transferred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each Pair	Proportion Red : Pink
			♀	♂	♀	♂				
A ..	45	27	604	578	89	81	1,282	170	1,452	7.5 : 1
B ..	19	15	169	145	30	41	314	71	385	4.4 : 1
C...	31	22	320	305	116	94	625	210	835	3.0 : 1
D ..	63	32	658	681	203	221	1,339	424	1,763	3.2 : 1
E ..	20	16	218	218	79	73	436	152	588	2.9 : 1
F...	47	28	476	435	153	144	911	297	1,208	3.0 : 1
G...	22	17	259	248	83	89	507	172	679	2.9+ : 1
H ..	32	22	392	396	152	125	788	277	1,065	2.8+ : 1
I...	63	32	864	805	258	281	1,669	539	2,208	3.1 : 1
J...	25	19	357	358	130	124	715	254	969	2.8 : 1

---

[5] Lived only eight days.

[6] The length of time a fly lived should not be taken as a criterion for measuring its vigor. In most cases death is accidental.

TABLE VIII

F_2 RECORD FROM RED-EYED FLIES CROSSED TO PINK-EYED FLIES MATED IN PAIRS. SHOWING THE TOTAL OUTPUT OF EACH F_1 PAIR

DURING ITS LIFETIME

RED ♀ × PINK ♂ → IN F_2

Pair	No. of Days Each Lived	No. of Times Transferred	Red		Pink		Total Red	Total Pink	Total No. Produced	Proportion Red : Pink
			♀	♂	♀	♂				
a...	28	20	366	294	124	112	660	236	896	2.8 : 1
b...	13	11	163	170	58	49	333	107	440	3.1 : 1
c...	7	5	74	90	22	28	164	50	214	3.2 : 1
d...	14	12	213	195	58	57	408	115	523	3.5+ : 1
e...	22	15	101	106	33	26	207	59	266	3.6 : 1
f...	21	16	215	266	95	78	481	173	654	2.8 : 1
g...	37	24	358	288	113	139	646	252	898	2.6 : 1
h...	45	27	433	444	179	169	877	348	1,225	2.2+ : 1
i...	43	26	546	533	187	185	1,079	372	1,451	2.9 : 1
j...	30	21	298	306	90	83	604	173	777	3.5 : 1

A comparison of Tables VI, VII and VIII suggests the possible presence of high and low pink-producing "strains" in these stocks. To test this, some of the offspring of pair *A* (Table VII) in which the ratio was 7.5 of red to 1 of pink, were inbred for the F_3 in order to see if the same ratio would persist. As there were among the red both homozygous and heterozygous forms, they were each mated to their pink sisters or brothers. This combination would give with the former all red (since red is dominant) and with the latter a ratio of 1:1.

Pair *F* (Table VII) in which the ratio was ideal, 3:1, was chosen for the control, and treated in like manner.

In this as in the preceding experiment the flies were mated in pairs and transferred to fresh bottles every second or third day. A peculiar thing happened. Out of 25 pairs taken from "*A*" only two gave offspring; the remaining 23 pairs were apparently sterile. It could not have been due to bad banana or any other unfavorable condition, for the flies had already been transferred five times and no pupæ were found in any of the other bottles. Furthermore, the 16 pairs of the control which ran parallel to them, and were fed with the same food, did well.

To find out whether these flies were actually sterile, each of the 14 remaining pairs—9 having meanwhile died—were separated and every individual mated to wild red-eyed stock. The sterility of the pink flies, both male and female, was found to be absolute, while all red of both sexes were fertile.

As an additional test, some of the offspring of the last cross were inbred *en masse* in order to extract the pink flies which they would produce, since some of them were heterozygous for eye color.

A small number of pink flies were obtained and mated to their red brother and sisters: each pink female was put in a bottle with 3 or 4 red males, and each pink male with 3 or 4 red females. Out of 19 individuals thus tested, only three were found to be fertile; the remaining 16 were sterile.

These facts seem to indicate that some factor or group of factors which make for sterility were present in the "pink"-containing gamete. The results are the more significant since the hybrid fly, in which this condition prevailed, produced a very low pink ratio. Of the control in which 10 pairs were found to be heterozygous for eye-color with an expectation of 1:1, the following results were obtained:

TABLE IX

RECORD OF THE OFFSPRING OF F_2 FLIES OF A CROSS OF PINK ♀ BY RED ♂ IN WHICH A RATIO WAS 3: 1, AS EXPECTED. (See pair *F* of Table VIII)

Red Heterozygous ♀ by (Brother) Pink ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	50	352	348	236	244	700	480	1,180	1.7 : 1
2	27	150	130	170	161	280	231	511	1.2 : 1
3	18	51	85	59	50	136	109	245	1.2+ : 1
4	29	251	245	240	258	496	498	994	1.0 : 1
5	10	147	132	133	112	279	245	524	1.1 : 1

The reciprocal cross gave the following:

Pink Heterozygous ♀ by (Brother) Red ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	20	82	89	69	59	171	128	299	1.3+ : 1
II	22	123	124	94	107	247	201	448	1.2 : 1
III	32	223	240	213	247	463	460	920	1.0 : 1
IV	29	215	176	184	163	391	347	738	1.1 : 1
V	40	216	178	101	136	394	237	631	1.7 : 1

It will be seen that all the red flies of the last cross were heterozygous, and should give, on further inbreeding, a ratio of 3:1. Four pairs out of five (one being sterile), taken from Pair III of Table IX, gave the following:

TABLE X

RED ♀ × RED ♂ OF PAIR III, TABLE IX

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
a....	40	312	348	43	45	660	88	748	7.5 : 1
b....	23	282	316	80	103	598	183	781	3.3 : 1
c....	40	194	214	60	70	408	130	538	3.1 : 1
d....	14	175	163	47	59	338	106	444	3.2 : 1

Pair "*a*" above gave the same result as pair "*A*" (Table VII)—the ratio in each case being 7.5:1. The offspring of the latter were found to contain a high percentage of sterile pink flies, owing to which the attempt then made to test that ratio failed. It was therefore decided to repeat the same experiment with the offspring of this "*a*" pair. As in the former case, the heterozygous red flies were picked out by crossing them to their pink brothers and sisters. The expectation was again 1:1. The records follow.

TABLE XI

RECORD OF EIGHT PAIRS, HETEROZYGOUS RED × TO PINK, TAKEN FROM THE OFFSPRING OF PAIR "*a*," TABLE X

Pink ♀ × Heterozygous Red ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
A...	27	179	176	79	88	355	167	522	2.1+ : 1
B...	12	155	153	83	66	308	149	457	2.0+ : 1
C...	28	163	205	126	159	368	285	653	1.3 : 1
D...	28	243	222	206	219	465	425	890	1.1 : 1

The reciprocal cross gave:

Red Heterozygous ♀ × *Pink* ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
a....	28	282	236	210	260	518	470	988	1.1 : 1
b....	28	296	290	5	5	586	10	596	59.0 : 1
c....	12	144	158	136	152	302	288	590	1.0+ : 1
d....	25	72	113	63	98	185	161	346	1.1+ : 1

The pair with which we started (*F*, Table VII) gave the ideal 3:1 ratio; but in each of the three generations which were bred from its offspring (Tables IX, X, XI) there appeared again the same fluctuations which were observed in the preceding experiments, and with even more striking emphasis. Among the offspring of the same pair are found some that give a 3:1 ratio and some that give a 7.5:1; in a second pair we have some giving 1:1, and one giving 59:1. The latter especially suggests the presence of a factor that actually inhibits the development of the pink flies, and, moreover, that it is being segregated in a mixed stock.

If the presence of such factor is assumed, we should be able by inbreeding to select stocks in which it is present and in which it is absent. For this reason the experiment recorded above (Table V) was here repeated with some modifications. It will be remembered that in the former, a fertilized red female and a fertilized pink male were placed in each bottle and their offspring, the F_1, counted. In the following experiment, in order to secure segregation, the F_2 were counted. Four virgin pairs, two red and two pink, were taken out of the culture bottles and mated separately. Of the F_1 of each of the four, six pairs were taken out, 24 pairs in all, and bred for the F_2. Unlike the first experiment, the males in this case were allowed to remain with the females throughout the experiment. This insured sufficient sperms for the eggs. Every second day the food was removed, together with the eggs deposited upon it, and fresh banana supplied. Each two batches of eggs—one deposited by a red female, one by a pink female—were placed together in one bottle so that they might develop side by side and under the same environmental conditions. The result of this experiment is shown in Table XII.

TABLE XII

F_2 of Two Pink Pairs *A, B,* and Two Red Pairs *a, b,* Showing Segregation of Productivity. Eggs of *A*I–*a*I, *B*I–*b*I, etc., Were Developed in the Same Bottle

Pink							Red						
	F_1 Pair	No. of Days Each Lived	♀	♂	Total Produced	Ave. Per Day		F_1 Pair	No. of Days Each Lived	♀	♂	Total Produced	Ave. Per Day
Pair *A*	*A* I	44	450	446	896	20.40	Pair *a*	*a* I	20	233	219	452	22.60
	A II	24	169	224	393	16.36		*a* II	22	9	8	17	0.77
	A III	28	61	67	128	4.57		*a* III	25	63	64	127	5.10
	A IV	30	221	233	454	15.13		*a* IV	31	272	232	504	16.26
	A V	36	331	345	676	18.77		*a* V	30	179	171	350	11.66
	A VI	21	37	33	70	3.33		*a* VI	22	152	138	390	17.73
Pair *B*	*B* I	29	199	217	416	14.34	Pair *b*	*b* I	44	122	104	226	5.10
	B II	24	93	93	186	7.75		*b* II	28	103	77	180	6.43
	B III	35	31	47	78	2.23		*b* III	44	302	256	558	12.70
	B IV	30	15	13	28	0.93		*b* IV	35	22	34	56	1.60
	B V	28	11	10	21	0.75		*b* V	31	302	293	595	19.20
	B VI	18	10	8	18	1.00		*b* VI	44	154	139	293	6.65

Segregation with respect to productivity is here evident. Whether the low fertility[7] seen in so large a proportion of these flies was due to an actually low egg-production, or whether it was due to something which prohibited development or to some defect in the germ cell owing to which fertilization could not be effected, is not known. That one of the latter possibilities is likely to be realized here can be inferred from the work of Dr. R. R. Hyde in this laboratory. He counted the eggs of hundreds of individuals, and compared them with the number of flies which emerged from them. According to his observations, only about 75 per cent. of the eggs of the wild fly ever reach maturity, and in some of the mutations no more than 25 per cent. of the eggs develop.

---

[7] The term "fertility" is used here, as defined by Hyde, to indicate the number of eggs that complete development and give rise to mature flies. (See Hyde, *Jour. Exp. Zool.*, August, 1914, p. 185.)

Another point of interest brought out in the last experiment is the fact that the wild, red-eyed fly behaves in exactly the same manner as the mutant pink fly. This may be the reason for the observed shifting of the ratio sometimes in favor of the one variety, sometimes in favor of the other. It shows furthermore that it was not the pink as such that caused the disturbance. The red also might be similarly disturbed and perhaps by the same agent or by another agent that affected the productivity in the same way.

If the abnormally low number produced by some of the pairs of Table XII be due to the inability of a large number of their eggs to develop, and if we assume this character to be transmissible, it must reappear in the F_2 of a cross in which one of the parents possessed this factor, *i. e.*, a large number of individuals, one quarter of the output, should fail to develop. This would be in accordance with Mendelian principles. A number of crosses were therefore made in various combinations with the individuals taken from Table XII. The results follow:

TABLE XIII

F_2 of 16 Pairs of a Cross Red by Pink in Which the Parents Came from *A*I, *a*I, Table XII, the Average Daily Productivity of Which Was 22 and 20, Respectively

A. Pink ♀ (Productivity 20 Per Day) × Red ♂ (Prod. 22 Per Day)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	17	301	294	110	104	595	214	809	2.80 : 1
2	32	484	457	160	147	941	307	1,248	3.01 : 1
3	32	504	570	181	189	1,074	370	1,444	2.90 : 1
4	32	559	548	205	200	1,107	405	1,512	2.73 : 1
5	8	125	126	40	35	251	75	326	3.35 : 1
6	18	261	243	98	89	504	187	691	2.70 : 1
7	21	391	449	155	142	840	297	1,137	2.86 : 1
8	32	673	700	203	236	1,373	439	1,812	3.10 : 1
Total number produced by 8 pairs						6,685	2,294	8,979	

Average proportion, 2.91 : 1

B. Red ♀ (Productivity 22 Per Day) × Pink ♂ (Prod. 20 Per Day)

Pair	No. of Days Each was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	30	415	395	135	127	810	252	1,062	3.21 : 1
II	14	132	121	41	73	253	114	367	2.22 : 1
III	24	317	337	97	94	654	191	845	3.42 : 1
IV	30	484	378	119	120	862	239	1,101	3.60 : 1
V	24	392	418	96	140	810	236	1,046	3.42 : 1
VI	30	656	592	198	200	1,248	398	1,646	3.14 : 1
VII	30	566	647	182	209	1,213	391	1,504	3.10 : 1
VIII	24	438	451	132	153	889	285	1,174	3.10 : 1
Total produced by 8 pairs..................						6,739	2,106	8,845	

Average proportion, 3.24 : 1

If the proportion of red to pink, realized in the F_2, depends upon the relative fertility of the two parents which form the cross, we should get in this case, where the parents were supposedly equally fertile, the ideal 3 : 1 ratio. The records, however, show considerable fluctuations. Nevertheless, these results are perfectly in accord with our hypothesis. Looking back to Table XII, which furnished the parents of this cross, the explanation is obvious. The averages per day for *A*I–*A*VI were 20, 16, 4, 15, 18 and 3, respectively. Similarly, *a*I–*a*VI gave 22, 0.7, 5, 10, 16, 11 and 17, respectively. It is therefore reasonable to assume that among the offspring of *A*I (productivity 20) and *a*I (productivity 22) individuals should be found which would repeat the series. Fluctuation is, therefore, to be expected. The average of many such pairs, however, should be 3 : 1. The proportion obtained was 2.91 : 1 in one case; 3.24 : 1 in the other, or a general average of 3.08 : 1.

It should also be noted here that in this as well as in the subsequent experiments, wherever eight pairs are recorded, they are not the offspring of one, but of two distinct crossings of one pair each which were made at the same time; that pairs 1–4, 5–8; I–IV, V–VIII, respectively, were brothers and sisters. More than one line is thus represented in each case. With these facts in mind, we may pass on to the remaining experiments.

TABLE XIV

F$_1$ OF 12 PAIRS OF A CROSS RED BY PINK IN WHICH THE PARENTS WERE *A*I AND *b*II (TABLE XII) THE AVERAGE DAILY PRODUCTIVITY OF WHICH WAS 20 AND 6, RESPECTIVELY

A. Pink ♀ (Productivity 20 Per Day) × Red ♂ (Productivity 6)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	32	355	331	117	95	686	212	898	3.23 : 1
2	28	340	373	126	132	713	258	971	2.76 : 1
3	19	330	288	120	124	618	244	862	2.52 : 1
4	32	544	562	188	188	1,106	376	1,482	2.94 : 1
5	23	574	578	203	214	1,152	417	1,569	2.76 : 1
6	14	196	194	66	59	390	125	515	3.12 : 1
7	32	648	603	222	246	1,251	468	1,719	2.67 : 1
8	32	429	374	141	144	803	285	1,088	2.81 : 1
Total number produced by 8 pairs.........						6,719	2,385	9,104	

Average proportion, 2.84 : 1

B. Red ♀ (Productivity 6 Per Day) × Pink ♂ (Productivity 20)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	28	488	482	176	177	970	353	1,323	2.74 : 1
II	6	83	78	21	30	161	51	212	3.15 : 1
III	28	463	465	164	153	928	317	1,245	2.93 : 1
IV	21	375	383	114	116	758	230	988	3.20 : 1
Total number produced by 4 years.......						2,817	951	3,768	

Average proportion, 2.96 : 1

In most of these pairs the pink slightly exceeded the 3:1 expectation. In the few in which they fell behind, the red (if we assume fertility to be the cause) might have been of a higher fertility than the pink, as has been explained. As a group, however, they give a proportion somewhat below 3:1.

In the next cross, the red fly was the more fertile. The results are given in the following table:

TABLE XV

F_2 OF A CROSS RED BY PINK IN WHICH THE PARENTS WERE *BIV* AND *aI* (TABLE XII) THE AVERAGE DAILY PRODUCTIVITY OF WHICH WAS 1 AND 22, RESPECTIVELY

A. Pink ♀ (Productivity 1 Per Day) × Red ♂ (Productivity 22)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	22	398	405	120	105	803	225	1,028	3.52 : 1
2	28	512	477	107	125	989	232	1,221	4.26 : 1
3	22	420	389	104	135	809	239	1,048	3.34 : 1
4	18	340	300	86	106	640	192	832	3.33 : 1
5	19	420	428	129	134	852	263	1,115	3.24 : 1
6	19	372	396	100	104	768	204	972	3.76 : 1
7	17	198	220	58	83	418	141	559	3.00 : 1
8	17	174	212	62	62	386	124	510	3.10 : 1
Total number produced by 8 pairs.........						5,665	1,620	7,285	

Average proportion, 3.50 : 1

B. Red ♀ (Productivity 22 Per Day) × Pink ♂ (Productivity 1)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	31	264	262	101	87	526	188	714	2.80 : 1
II	27	353	350	80	85	703	165	868	4.26 : 1
III	31	430	403	97	112	833	209	1,042	4.00 : 1
IV	31	427	420	111	128	847	239	1,086	3.54 : 1
V	28	486	471	122	147	957	269	1,226	3.60 : 1
VI	28	573	568	139	154	1,141	293	1,434	3.90 : 1
VII	28	505	505	116	127	1,010	243	1,253	4.15 : 1
VIII	24	502	501	134	140	1,003	274	1,277	3.66 : 1
Total produced by 8 pairs..................						7,020	1,880	8,900	

Average proportion, 3.73 : 1

Of the 16 pairs of this cross only one gave less than 3 : 1. In the remaining 15, the proportion was, in each case, considerably higher than 3 : 1. It will be noted that of all 16 pairs that one was the least fertile. This would indicate, on the hypothesis suggested, that the gamete containing the "red" factor did not have relatively as high a potential of fertility as did the parent which produced it.

A comparison of Tables XIV and XV shows that we have two distinct groups: one in which the extracted pink exceed the expectation, and one in which they fall behind the expectation. Yet the method employed in each case was the same; the history of each is the same. The only difference is to be found in the fact that in the one case the pink came from a more fertile parent; in the other, the red.

The offspring of pairs "7" (Table XIV) and "2" (Table XV) in which the ratios were 2.67:1 and 4.26:1, respectively, were inbred for the F_3. Fifteen pairs were taken from each, but as there were among the red both homozygous and heterozygous flies, only eight gave pink in each case. The results follow:

TABLE XVI

RECORD OF 8 PAIRS HETEROZYGOUS RED-EYED F_2 OF PAIR "7" (TABLE XIV) IN WHICH THE RATIO WAS 2.67: 1

Pair	No. of Days	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	14	270	279	80	78	549	158	707	3.47 : 1
2	14	159	167	58	42	326	100	426	3.26 : 1
3	14	5	7	2	1	12	3	15	4.00 : 1
4	14	135	143	46	49	278	95	373	2.93 : 1
5	14	183	157	49	50	340	99	439	3.23 : 1
6	14	172	154	45	42	326	87	413	3.74 : 1
7	14	142	137	43	49	279	92	371	3.00 : 1
8	14	57	46	12	16	103	28	131	3.67 : 1

TABLE XVII

RECORD OF 8 PAIRS HETEROZYGOUS RED-EYED F_2 OF PAIR "2" (TABLE XV) IN WHICH THE RATIO OF RED TO PINK WAS 4.26: 1

Pair	No. of Days	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	14	295	308	100	97	603	197	800	3.06 : 1
2	14	392	326	97	105	718	202	920	3.55 : 1
3	14	281	288	95	86	569	181	750	3.14 : 1
4	14	110	104	32	33	214	65	279	3.31 : 1
5	14	167	170	70	59	337	129	466	2.61 : 1
6	14	314	277	82	86	591	168	759	3.52 : 1
7	14	160	169	32	41	329	73	402	4.50 : 1
8	14	133	146	49	41	279	90	369	3.10 : 1

These results are significant, in that they show that the original ratios, which their parents gave, were lost.

The fact that it has been found possible by proper manipulation to get a group in which the ratio fluctuated in one direction only, even if it was not as marked as was hoped it would be, indicates that the disturbance is due to an *internal,* and not to an external, cause. This was further emphasized by the distinct tendency for segregation, as was to be expected if there were some heterozygous individuals. It was also suggested in another way. No matter how short-lived or how long-lived a pair was; whether it was transferred once, twice, or even twenty times, the ratio of red to pink did not vary throughout its life when the yields of the several bottles were compared with one another.

TABLE XVIII

F_2 OF FOUR PAIRS (*A, B, C* AND *D*) RED ♀ × PINK ♂ IN WHICH THE MALES WERE CROSSED EACH TO SEVERAL OF HIS OWN DAUGHTERS

Expectation 1 : 1

Pink ♂	No. of ♀	Total Red	Total Pink	Proportion Red : Pink
A ×	1	97	45	2.15 : 1
	2	105	71	1.46 : 1
	3	231	161	1.43 : 1
	4	171	122	1.40 : 1
	5	211	194	1.10 : 1
B ×	1	110	80	1.38 : 1
	2	263	251	1.00 : 1
	3	242	198	1.28 : 1
	4	183	143	1.28 : 1
	5	231	189	1.22 : 1
	6	162	102	1.62 : 1
C ×	1	201	113	1.78 : 1
	2	257	116	2.22 : 1
	3	255	206	1.27 : 1
	4	206	153	1.36 : 1
	5	243	197	1.23 : 1
	6	125	98	1.27 : 1
D ×	1	179	135	1.30 : 1
	2	217	121	1.80 : 1
	3	107	74	1.44 : 1

TABLE XIX

F_2 OF PAIRS *A, B, C* AND *D* OF TABLE XVIII

Expectation 3 : 1

F_1 of Pair	No. of Pair	Total Red	Total Pink	Proportion Red : Pink
A	1	153	41	3.73 : 1
	2	158	55	3.00 : 1
	3	96	21	4.60 : 1
B	1	140	43	3.27 : 1
	2	215	72	3.00 : 1
	3	136	71	1.92 : 1
C	1	50	21	2.38 : 1
	2	125	43	2.90 : 1
	3	122	49	2.49 : 1
	4	193	67	2.88 : 1
D	1	26	6	4.33 : 1
	2	112	34	3.30 : 1
	3	194	74	2.62 : 1

The proportion of red to pink was found to bear a direct relation to the relative "fertility" of the parents which produced the hybrid. This suggests a causal relation between the two.

In dealing with "fertility" the difficulty that one encounters is, that the offspring of any pair may, with respect to this character, differ from either parent, and also differ amongst themselves, forming a graded series running from the most to the least fertile. An individual taken from such a population is an indefinite quantity and will often defeat the purpose of the experiment. In order to simplify this as far as possible, the following experiment was planned:

Four red-eyed, virgin females were each mated to a pink male. Each male was again crossed to several of his own daughters. The records are given in Table XVIII. As a control a number of F_1 pairs were bred in each case. The records are given in Table XIX.

A graphic representation of all pairs recorded in Tables VI–XIX, except for the several very unusual ratios, is given in Figs. 1 and 2.

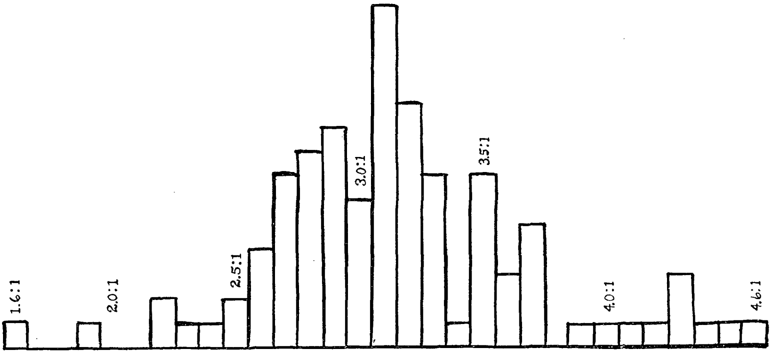

Fig. 1

Fig. 1 contains 99 pairs in each of which the expected ratio was 3:1, with a total population of 82,607. They are distributed as follows:

No. of Pairs		Ratios of Red : Pink
1	gave	1.6 : 1
0	gave	1.7 : 1
0	gave	1.8 : 1
1	gave	1.9 : 1
0	gave	2.0 : 1
0	gave	2.1 : 1
2	gave	2.2 : 1
1	gave	2.3 : 1
1	gave	2.4 : 1
2	gave	2.5 : 1
4	gave	2.6 : 1
7	gave	2.7 : 1
8	gave	2.8 : 1
9	gave	2.9 : 1
6	gave	3.0 : 1
14	gave	3.1 : 1
10	gave	3.2 : 1
7	gave	3.3 : 1
1	gave	3.4 : 1
7	gave	3.5 : 1
3	gave	3.6 : 1
5	gave	3.7 : 1
0	gave	3.8 : 1
1	gave	3.9 : 1
1	gave	4.0 : 1
1	gave	4.1 : 1
1	gave	4.2 : 1
3	gave	4.3 : 1
1	gave	4.4 : 1
1	gave	4.5 : 1
1	gave	4.6 : 1
Total . . 99		

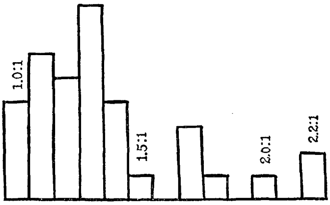

Fig. 2

Fig. 2 contains 37 pairs of back-crosses; expected ratio 1 : 1; total population 17,008. They are distributed as follows:

No. of Pairs		Ratios of Red : Pink[8]
4	gave	1.0 : 1
6	gave	1.1 : 1
5	gave	1.2 : 1
8	gave	1.3 : 1
4	gave	1.4 : 1
1	gave	1.5 : 1
1	gave	1.6 : 1
3	gave	1.7 : 1
1	gave	1.8 : 1
0	gave	1.9 : 1
1	gave	2.0 : 1
1	gave	2.1 : 1
2	gave	2.2 : 1
Total. . 37		

To these should be added:

1 pair which gave 9.7 : 1, Table V.
1 pair which gave 6.5 : 1, Table VI
1 pair which gave 7.5 : 1, Table VII
1 pair which gave 7.5 : 1, Table X
1 pair which gave 59.0 : 1, Table XI

Except for the several detached pairs at the extreme limits, Fig. 1 shows a normal curve. A disturbance of 0.5 in either direction (less than 10 per cent.) is quite within the limits of experimental accuracy. The larger disturbances, ratios of 6 or 7 : 1, and also the first results reported by Morgan ('11 and '12) are yet to be explained. These are too large to be attributed to experimental error.

The data presented in the foregoing pages show that there has been a marked improvement in the ratio of pink to red since 1911. In one case only (1913) was the disturbance greater than those of Morgan (59 : 1). The remaining very marked disturbances were between 6 and 10 : 1. And these appeared so infrequently that in mass-cultures their presence would hardly have been felt.

A corresponding improvement has also been observed in the fertility of the pink-eyed race between 1912 and 1913. This is seen on comparing Tables V and XII. In the first, the fertility of the pink was much lower than that of the red; in the second (about one year later), it was as high.

Hyde ('14) showed that in some races of *Drosophila*

---

[8] None gave a ratio of less than one red to one pink.

ampelophila, the number of eggs failing to reach maturity is between 25 per cent. and 75 per cent. of the total output; and concluded that this peculiarity probably behaves as a Mendelian recessive factor. More recently, Morgan ('14) describes recessive lethal factors in *Drosophila,* which he defines, "as any factor that brings about the death of the individual in which it occurs, provided that its effect is not counteracted by the action of its normal allelomorph."

In the light of this evidence, the following conclusions suggest themselves:

1. The original pink-eye mutant was heterozygous for some non-sex-linked factor which, in the homozygous state, acts like Morgan's lethal. This factor was, in the course of time, to a large extent eliminated, as is to be expected if the individuals homozygous for it are more likely to die. The chance of such homozygous forms appearing again, has thereby been much reduced. This is borne out by, and also explains, the improvement in the pink race.

2. A similar recessive, though not necessarily the same factor, might also be present in some individuals of the wild, red-eyed stock. Hyde's work mentioned above gives weight to this assumption—which is not at all an unreasonable assumption in a species as unstable as this, judging by the vast number of mutations reported. For this reason, the red sometimes fall behind the expected ratio.

3. The mode of action of these lethals shows that they are linked to the "pink" factor or to its normal "red" allelomorph. This will be clear from the following analysis:

Of the flies recorded in Fig. 2, one parent was *RP* (with gametes *R* and *P*); the other was *PP* (with gametes *P* and *P*). The zygotes resulting from these gametes almost invariably give fewer *PP*'s than *RP*'s. In other words, the homozygous forms run behind the heterozygous forms. The relation between these two classes may also be supposed to hold in the F_2 cross (Fig. 1). Here, however, the reds (*RR* and *RP*) run relatively less often ahead of

PP. This must be due to a deficiency in the homozygous *RR* flies. In other words, the results taken all together (Figs. 1 and 2) show that the disturbance is brought about by factors (in the third chromosome) which in the homozygous state act as lethals or perhaps as semi-lethals. Random introduction of one or two or no lethals may be assumed, as follows:

(*A*) If the lethal is introduced by the "pink-bearing" chromosome, the homozygous pink will be depressed in the F_2.

(*B*) If introduced by the "red-bearing" chromosome, the homozygous red will be depressed in the F_2.

(*C*) If two lethals, both of which are identical, are introduced at the same time, one by the red and one by the pink, all classes will be equally depressed,[9] and the results as far as concerns the F_2 ratio will be the same as if there were no lethals present, *i. e.*, the 3:1 ratio will be realized.

(*D*) If two lethals that are different are introduced at the same time, one by the red and one by the pink, both the homozygous classes (*RR* and *PP*) will be depressed, but not the *RP*. There would be somewhat fewer pinks than expected in the F_2.

I wish to acknowledge my indebtedness to Professor T. H. Morgan, whose kind attention and suggestions both throughout the foregoing experiments and in the preparation of the present report, were invaluable. I also wish to express my appreciation to Mr. H. J. Muller to whom I owe some suggestions concerning the interpretation of the results.

LITERATURE CITED

Hyde, R. R. Fertility and Sterility in *Drosophila ampelophila*. *Jour. Exp. Zool.*, Vol. 17, No. 2, August, 1914.

Morgan, T. H. An Attempt to Analyze the Constitution of the Chromosomes on the Basis of Sex-Limited Inheritance in *Drosophila*. *Jour. Exp. Zool.*, Vol. 11, No. 4.

Two Sex-Linked Lethal Factors in *Drosophila* and their Influence on the Sex-Ratio. *Jour. Exp. Zool.*, Vol. 17, No. 1, July, 1914.

---

[9] "Crossing-over" is ignored as the character of the results is not changed thereby.